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Intramyocellular Lipid Content and Molecular Adaptations in Response to a 1-Week High-Fat Diet

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Abstract

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Objective: To investigate molecular adaptations that accompany the elevation of intramyocellular lipid (IMCL) content on a high-fat (HF) diet for 1 week.

Research Methods and Procedures: Ten subjects consumed a normal-fat (NF) diet for 1 week, followed by an HF diet for another week. After both dietary periods, we determined the IMCL content by proton magnetic resonance spectroscopy in the vastus lateralis muscle and quantified changes in gene expression, protein content, and activity in biopsy samples. We investigated genes involved in carbohydrate and fatty acid handling [lipoprotein lipase, acetyl-coenzyme A carboxylase (ACC) 2, hormone-sensitive lipase, hexokinase II, and glucose transporter 4] and measured protein levels of CD36 and phosphorylated and unphosphorylated ACC2 and the activity of adenosine monophosphate-activated kinase.

Results: IMCL content was increased by 54% after the HF period. Lipoprotein lipase mRNA concentration was increased by 33%, whereas ACC2 mRNA concentration tended to be increased after the HF diet. Hexokinase II, glucose transporter 4, and hormone-sensitive lipase mRNA were unchanged after the HF diet. ACC2 and CD36 protein levels, phosphorylation status of ACC2, and adenosine monophosphate-activated kinase activity did not change in response to the HF diet.

Discussion: We found that IMCL content in skeletal muscle increased after 1 week of HF feeding, accompanied by molecular adaptations that favor fat storage in muscle rather than oxidation.

Key words: human, intramuscular triglycerides, dietary fat, skeletal muscle, magnetic resonance spectroscopy

Introduction

High-fat (HF)[1] diets, which are widely consumed in western societies, have been related to the development of chronic illnesses and obesity (1). Unlike carbohydrate and protein, fat does not stimulate its own oxidation, and it is well known that the adaptation of fat oxidation is slow (2,3), favoring fat storage when subjects switch from a low-fat to an HF diet. Although the major part of the excessive fat will be stored in white adipose tissue, triglyceride content in skeletal muscle has also been reported to increase after HF feeding (4–8). With proton magnetic resonance spectros-

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[1] Nonstandard abbreviations: HF, high fat; ^{1}H-MRS, proton magnetic resonance spectroscopy; IMCL, intramyocellular lipid; VLDL, very low-density lipoprotein; NF, normal fat; FFA, free fatty acid; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; HKII, hexokinase II; GLUT4, glucose transporter 4; ACC, acetyl-coenzyme A carboxylase; RT, reverse transcription; PCR, polymerase chain reaction; AU, arbitrary unit(s); AMPK, adenosine monophosphate-activated kinase.

copy (^{1}H-MRS), separation between intramyocellular lipid (IMCL) content and contaminating adipose tissue (extramyocellular lipids) can be made, and IMCL can be quantified in a volume of a few cubic centimeters. Using this method, so far only the very acute effect of HF diets (2 to 3 days) has been investigated (5,6). Here, we aimed to investigate IMCL content in m. vastus lateralis by ^{1}H-MRS after 1 week of an HF diet with a macronutrient composition similar to our previous work, in which we determined the effect of an HF diet on fatty acid oxidation (see below) (3).

An increase in IMCL content, as observed on an HF diet, could be simply the consequence of a positive fat balance, due to the slow adaptation of fat oxidation to increased fat intake. However, it is interesting to note that endurance training also increases IMCL content (9), which is not due to a positive fat balance. With training, the oxidation of non-plasma-derived fatty acids [very low-density lipoprotein (VLDL) and/or IMCL] is also increased (10,11), suggesting that the increase in IMCL serves to increase IMCL oxidation. Indeed, some authors found intramuscular triglyceride content to be positively related to IMCL use during exercise (6,7,12,13). Therefore, instead of being simply the result of a positive fat balance, an alternative explanation could be that an increased content of IMCL on an HF diet could lead to increased IMCL breakdown and, therefore, increased availability of intramuscular lipid substrate. In this context, we previously showed that whole-body fat oxidation was increased after 1 week of an HF diet and that the increase in fat oxidation was completely accounted for by an increased oxidation of VLDL and/or IMCL (14).

Therefore, to examine whether the increase in IMCL content on an HF diet is simply due to a positive fat balance or serves to increase fat oxidation, we investigated molecular markers of lipid storage and oxidation in skeletal muscle. We recently investigated the same molecular markers after a short-term endurance training period, which was shown to result in a 42% increase in IMCL content (15) and found a 29% decrease in ACC2 mRNA expression, indicating an improved fat oxidative capacity (V. Schrauwen-Hinderling, unpublished data). In the present study, we quantified IMCL content by ^{1}H-MRS after 1 week of normal-fat (NF) diet and 1 week of HF diet, and we investigated mRNA and protein levels and protein activity in muscle biopsies at the end of both dietary periods.

Research Methods and Procedures

Subjects

Ten young and healthy male subjects [age (years) (mean ± SD), 25.0 ± 6.2] participated in this study. Subjects were recruited by advertisement, and the study was approved by the institutional Medical Ethics Committee. Subjects gave their written informed consent after the nature of the procedure was explained.

Experimental Protocol

Subjects were provided with the experimental diet for 1 week at a time (NF diet and subsequently HF diet). In the morning of the 7th day, a biopsy was taken in the fasted state, and in the afternoon of the same day, a ^{1}H-MRS scan was performed to determine the IMCL content in the m. vastus lateralis. To exclude acute effects of exercise on IMCL content, subjects refrained from exercise the 2 days preceding the ^{1}H-MRS measurements.

Diets

The macronutrient composition of the NF diet was such that 30% of the energy was consumed as fat, 55% as carbohydrate, and 15% as protein. In the HF diet, 60% of the energy was consumed as fat, 25% as carbohydrate, and 15% as protein.

According to the self-reported physical activity level, subjects were given 1.6 to 1.7 times their basic metabolic rate [based on Harris and Benedict equations (16)]. Additionally, subjects received snacks with the same macronutrient composition as the whole diet, which they could eat if needed. The diet consisted of all normal ready-to-use food. Every subject received a personal schedule for every day, listing the provided food items for breakfast, lunch, dinner, and snacks.

Procedures

MRS Measurement. Image-guided localized single-voxel ^{1}H-MRS was performed as described earlier (15) in the m. vastus lateralis after the NF and the HF dietary period. MRS measurements were performed on only eight subjects, and for one of them, the spectra were of insufficient quality to be analyzed reliably. Therefore, IMCL content is reported from seven subjects.

Post-processing. The spectra were fitted in the time domain using a non-linear least-squares algorithm [AMARES (17)] in the jmrui software package (18) (http://www.mrui.uab.es/mrui/) using prior knowledge as described earlier (15). Six peaks were fitted in total, namely three peaks for extramyocellular lipids and three peaks for IMCL. The signals were corrected for T1 and T2 relaxation using the T1 and T2 relaxation times as determined by Schick et al. (19). The corrected area of the CH_2 peak of IMCL was expressed relative to the area of the corrected water peak. The reproducibility of the quantification of IMCL in the m. vastus lateralis was determined in the setting of another study and reported earlier (15).

Body Weight. Body weight was measured in the morning in the fasted state after both dietary periods on a digital scale (E1200; Sauter, Albstadtl-Elbingen, Germany) with an accuracy of 0.01 kg.

Analysis

Blood Analyses. For the determination of free fatty acids (FFAs) and triacylglycerol plasma concentrations, blood

was collected in tubes containing 30 μl of 0.2 M EDTA. Plasma was immediately centrifuged at high speed, frozen in liquid nitrogen, and stored at −80 °C for later analyses. Triacylglycerol (GPO-trinder 337B; Sigma Diagnostics, St. Louis, MO) and FFA (Wako NEFA-C kit, Wako Chemicals, Neuss, Germany) concentrations were analyzed using the COBAS FARA semiautomatic analyser (Roche, Basel, Switzerland).

Muscle Biopsy Sampling and Analysis. Muscle biopsies were taken from the midthigh region of m. vastus lateralis according to the technique of Bergström (20). The biopsy was used for isolation of total RNA using the acid phenol method of Chomozynski and Sacchi (21), with concomitant acid phenol extraction and ethanol precipitation. The mRNA concentrations of lipoprotein lipase (LPL), hormone-sensitive lipase (HSL), hexokinase II (HKII), glucose transporter 4 (GLUT4), and acetyl-coenzyme A carboxylase (ACC) 2 were quantified by reverse transcription (RT)-competitive polymerase chain reaction (PCR) (22). For the assays, the RT reaction was performed from 0.2 μg of skeletal muscle total RNA in the presence of a thermostable reverse transcriptase (Tth; Promega, Madison, WI) by use of one of the specific antisense primers. The competitive PCR assays were performed as previously described (23–25). To improve the quantification of the amplified products, fluorescent dye-labeled sense oligonucleotides were used. The PCR products were separated and analyzed on an ALF express DNA sequencer (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) with the Fragment Manager Software. Total RNA preparations and RT-competitive PCR assays of both skeletal muscle samples from the same individual (after NF and HF diets) were performed simultaneously. The mRNA data are reported in arbitrary units (AUs), and the average of the baseline value is set to 1. CD36 protein levels were determined by Western blotting, using a monoclonal antibody (MO25) as previously described (26). Phosphorylated and unphosphorylated ACC2 protein levels were determined as previously described (27). ACC2 protein levels are reported as the ratio of ACC2 to actin, whereas the phosphorylation status of ACC2 is reported as the ratio of phosphorylated to unphosphorylated ACC2 protein. Adenosine monophosphate-activated kinase (AMPK) activity has been investigated as described earlier (27). Total AMPK activity was measured in the tissue lysates by immunoprecipitating with a combination of α 1- and α 2-specific antibodies. A peptide kinase activity could be detected in immunoprecipitates using a variation of the method of Davies et al. (28). Assays were conducted using the AMARAASAAALARRR peptide (29). Activities are presented in units per milligram protein, where unit is defined as nanomoles of phosphate incorporation into the AMARA peptide per minute.

Statistics. Results are reported as mean ± SE. Statistical analyses were performed with SPSS for Windows 10.0.0 software (SPSS Inc., Chicago, IL). Differences after the two dietary periods were detected with paired Student's *t* tests. Results were considered significant if $p < 0.05$.

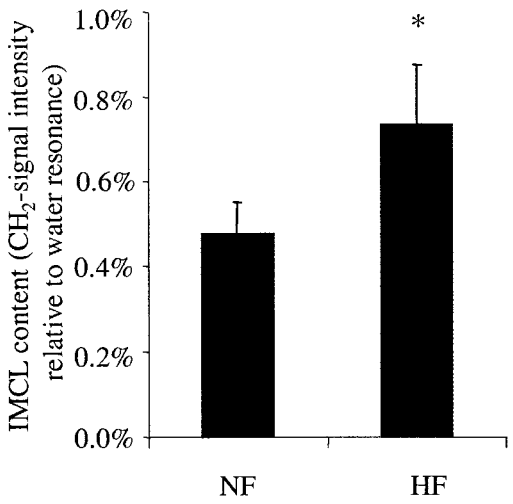

Figure 1: IMCL content after the NF and HF dietary periods ($n = 7$). The IMCL content was significantly higher after the HF diet ($p = 0.03$). * Significantly different from the NF dietary period.

Results

Energy Intake and Body Weight

By design, energy intake during the two dietary periods was identical [13.7 ± 0.7 MJ/d vs. 13.7 ± 0.7 MJ/d on the NF and the HF diets, respectively (not significant)]. The fat intake was 29.7 ± 0.2% and 60.1 ± 0.2% of total energy intake on the NF and HF diets, respectively. Body weight was unaffected by the type of diet [80.1 ± 4.4 and 79.9 ± 4.6 kg after the NF and HF diets, respectively (not significant)].

IMCL

IMCL content was significantly increased by 53.8% after the HF period, compared with the NF period (0.48% ± 0.07% of the water resonance after the NF diet vs. 0.73% ± 0.15% after the HF diet, see Figure 1). If values were converted to values in millimoles per kilogram of muscle wet weight as described by Boesch et al. (30) IMCL content was 6.12 ± 0.94 and 9.4 ± 1.87 mmol/kg after the NF and HF diets, respectively.

mRNA

Concentrations of HKII (1.00 ± 0.24 vs. 0.90 ± 0.20 AU after NF and HF diets, respectively), Glut4 (1.00 ± 0.32 vs. 1.00 ± 0.25 AU after NF and HF diets, respectively) and HSL mRNA (1.00 ± 0.26 vs. 1.49 ± 0.48 AU after NF and HF diets, respectively) were not different after the two dietary periods. LPL mRNA concentration was increased

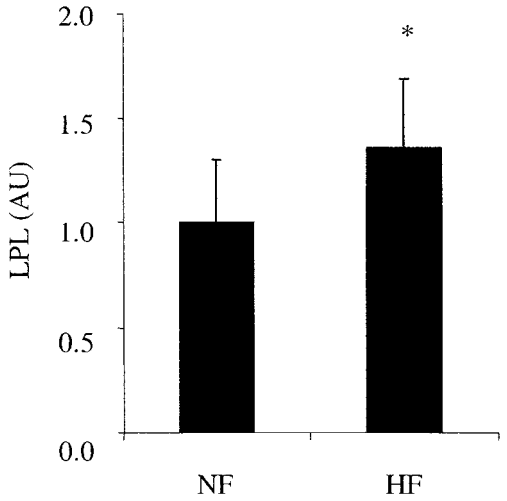

Figure 2: LPL mRNA concentrations increased significantly after the HF dietary period compared with the NF dietary period ($p = 0.04$). * Significantly different from the NF dietary period.

significantly by 36% (Figure 2), and ACC2 mRNA tended to be increased by 33% after the HF dietary period (1 ± 0.22 vs. 1.33 ± 0.24 AU after NF and HF diets, respectively, $p = 0.08$).

Protein Levels and Activities

ACC2 protein level (ACC2/actin) was not different after the HF diet (1.07 ± 0.14 vs. 1.02 ± 0.24 AU after NF and HF diets, respectively). The phosphorylation status (pACC2/ACC2) indicating the activation of the protein did not change either and was 2.97 ± 0.47 vs. 3.33 ± 0.50 AU after the NF and the HF diets, respectively (ratio of the signals obtained with the phosphospecific antibody and streptavidin). AMPK activity was not different after the two dietary periods (0.0136 ± 0.0022 and 0.0173 ± 0.0054 U/mg after NF and HF diets, respectively). The CD 36 protein level was not different after the two dietary periods (88 ± 11 AU after NF diet vs. 87 ± 12 AU after HF diet).

Plasma Parameters

Fasting FFA concentrations increased after the HF diet compared with the NF diet, but there was no difference in triacylglycerol concentration after the two diets (see Table 1).

Table 1. Plasma concentrations of FFAs and triacylglycerols

	Normal-fat diet	HF diet
FFAs (μM)	251 ± 42	334 ± 54*
Triacylglycerols (μM)	961 ± 136	733 ± 175

FFA, free fatty acid; HF, high fat.
* Significantly different from normal-fat diet ($p = 0.02$).

Discussion

In contrast to carbohydrates, the intake of fat does not acutely stimulate its own oxidation, and consumption of an HF diet often leads to a positive fat balance. In addition to white adipose tissue, fat can also be stored in non-adipose tissues, such as heart, liver, and muscle. Here, we show that after 1 week of HF feeding, IMCL content of the vastus lateralis muscle was significantly increased. It can be suggested that increased IMCL content on an HF diet is simply the result of the positive fat balance that inevitably occurs when switching to an HF diet. Alternatively, it can be suggested that the increased IMCL content on the HF diet could lead to an increase in fat oxidation. Therefore, we examined in the present study the expression of genes involved in lipid handling in skeletal muscle and protein content and activity in response to an HF diet of 1 week.

The reported increase in IMCL content in the present study (54%) is well in line with earlier results in a range of muscles and with different methodologies [36% to 57% increase in IMCL content after 2 to 3 days of an HF diet (5–7) and 86% to 130% increase after 4 to 5 weeks (8,31)]. When the increased IMCL content on an HF diet serves to allow increased IMCL oxidation, an up-regulation of HSL would be anticipated. HSL is necessary for the hydrolysis of intramuscular lipid to allow mitochondrial uptake and subsequent oxidation (32,33). To the best of our knowledge, no data are available on the effect of HF feeding on HSL mRNA expression or activity in human skeletal muscle. Presently, we found that HF feeding did not affect skeletal muscle HSL mRNA expression.

Furthermore, we examined the effect of HF feeding on ACC2 mRNA and protein expression, as well as ACC2 phosphorylation and AMPK activity. ACC2 has been reported to play a critical role in regulating fat oxidation in skeletal muscle. Its unphosphorylated form catalyzes the carboxylation of acetyl-coenzyme A to form malonyl-CoA, an intermediate that inhibits the activity of carnitine-palmitoyl-transferase-1. Carnitine-palmitoyl-transferase-1 catalyzes the rate limiting step in the transfer of fatty acids into mitochondria, where they undergo oxidation. Therefore, a reduction in ACC2 or an increase in the phosphorylation of the protein would increase fat oxidation. Indeed, it was shown that ACC2 knockout mice are characterized by a strongly increased fat oxidation (34). Similarly, fat oxidation is stimulated when the activity of AMPK, which phosphorylates ACC2, increases. In humans, we have previously shown that a 3-month training program resulted in an increase in triacylglycerol (IMCL and/or VLDL)-derived fat oxidation and a reduction in ACC2 mRNA expression (11).

Therefore, when the increased IMCL content on the HF diet would serve to allow increased fat oxidation, we anticipated finding a reduction in ACC2 mRNA and protein level and/or increased phosphorylation of the protein after 1 week of HF feeding. In the current study, however, we report a tendency ($p = 0.08$) to increased ACC2 mRNA concentrations and no change in protein level, rather than the anticipated decrease. Also, AMPK activity and the phosphorylation of its downstream target, ACC2, were unaffected after the HF dietary period. This again suggests that it is unlikely that the increase in IMCL content on an HF diet serves to increase fat oxidation but points toward a role for IMCL in storing excessive fat when consuming an HF diet. This might explain the negative effect of HF diet on insulin sensitivity (35) because IMCL content has been positively associated with the development of insulin resistance (36) and is increased in patients with type 2 diabetes (37,38). However, we have recently shown that 2 weeks of endurance training resulted in a 42% increase in IMCL content, without any detrimental effect on insulin sensitivity (15). Interestingly, the increase in IMCL content was accompanied by a down-regulation of ACC2 mRNA expression (V. Schrauwen-Hinderling, unpublished data). This points toward an important role for ACC2 in determining the effect of IMCL on insulin sensitivity and suggests that high IMCL content does not affect insulin sensitivity as long as it is combined with improved fat oxidative capacity, as was also recently suggested by Goodpaster et al. (37). Interestingly, it was recently shown that HF feeding of wild-type mice resulted in the development of a diabetic phenotype, whereas ACC2 knockout mice were resistant against HF diet-induced obesity and diabetes (34). Therefore, ACC2 seems to be an important target in the treatment of type 2 diabetes and obesity.

An alternative source for fat oxidation could be VLDL. Helge et al. (4) showed that on an HF diet, VLDL oxidation was significantly increased during aerobic exercise. Triacylglycerols from VLDL particles have to be hydrolyzed by LPL located on the endothelial cells lining the blood vessels to be taken up by the skeletal muscles. Because the availability of triacylglycerols increases on an HF diet, a feasible response would be an increased expression of LPL. Indeed, we found the LPL mRNA concentration to be increased by 36% after the HF diet. The increase in LPL suggests that more fatty acids from VLDL are taken up into myocytes.

Although FFA can enter the cells by passive diffusion (39), facilitated diffusion has been described, and proteins with a high fatty acid binding affinity, such as CD36, have been characterized (40). It has been shown that increased fatty acid uptake may result in increased lipid accumulation in muscle (41). Therefore, we hypothesized that after the HF diet, the increased fat availability would result in increased levels of CD36, a key fatty acid translocase. We did, however, not find changes in the protein level of CD36 after increased dietary fat. In contrast, in trained athletes, CD36 was increased after an HF diet (42). Because training profoundly improves fat oxidative capacity and, hence, warrants increased trans-sarcolemmal transport of fatty acids, the discrepancy could be due to the differing training status of the subjects in the two studies.

To investigate whether 1 week of an HF diet affected genes involved in glucose metabolism, we investigated markers of carbohydrate metabolism (HKII and GLUT4). However, these markers did not change in response to the HF diet. Because the time course of the adaptations to an HF diet of the genes under investigation is presently unknown, it cannot be excluded that transient changes in mRNA might have been missed in the present investigation. However, the protein data reported here are in line with the lack of effect on fat oxidative capacity after the 1-week dietary intervention.

In conclusion, we investigated the adaptive response to an HF diet, which is currently associated with an unhealthy lifestyle. We found that IMCL content was increased after 1 week of HF feeding, which was not accompanied by molecular adaptations that improve fat oxidative capacity.

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